Appendix A

Version With Markings to Show Changes Made

1. A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

(i) incubating the nucleic acid sample with a primer, DNA polymerase and a deoxynucleotide tripohsphate [or the corresponding] deoxynucleotide triphosphate analogue or a dideoxynucleotide triphosphate [(representing a single base?)];

(ii) measuring the pyrophosphate released in step (i); and

(iii) identifying the nature of the nucleotide base added by measuring which nucleotide caused the release of pyrophosphate (Ppi) in step (ii)

wherein [characterised in that] steps (i) to (iii) are performed in a microfluidic device.

2. A method for identifying the sequence of a portion of sample DNA [which methodcomprises] comprising the steps of:

(i) forming immobilised double stranded DNA on one or more reaction areas in a microchannel structure of a microfluidic device[:];

(ii) adding a [known] deoxynucleotide, [(or the corresponding] deoxynucleotide analogue, or dideoxynucleotide[)] and a DNA polymerase to each of said one or more reaction areas so that extension of primer only occurs if there is a complementarity of the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the strand of sample DNA that is part of the immobilised double stranded DNA;

(iii) detecting whether or not the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added in step (ii) has been added to the primer DNA in said one or more reaction areas[,]; and

(iv) repeating steps (ii) and (iii) as required with a different deoxynucleotide, [(or the corresponding] deoxynucleotide analogue or dideoxynucleotide[)].

3. A method of determining a nucleotide base in a nucleic acid sample [according to claim 1 or 2] comprising the steps of:

(i) attaching 0.1 – 200 pmol of a primer or single stranded DNA sample to each of between one and 100,000 pre-determined areas on the surface of a microfluidic device;

(ii) hybridising small amounts[, e.g. 0.1-200 pmol,] of single stranded sample DNA or primer respectively to each of the predetermined areas;

(iii) adding a [known] deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase so that extension of the primer only occurs, with consequent release of pyrophosphate [(Ppi)], if there is a complementarity with the sample DNA;

(iv) measuring the release of pyrophosphate [Ppi] and from which predetermined area on the device it is released; and

(v) repeating steps (iii) and (iv) as required to construct a DNA sequence for the elongated primers, and hence for portions of the sample DNA.

4. A method for identifying the sequence of a portion of sample DNA, [which method comprises] comprising the steps of:

(i) adding sample DNA to a predetermined area on a microfluidic device;

(ii) moving the sample to a reaction chamber on the microfluidic device;

(iii) attaching the sample DNA to a surface of the reaction chamber [alternatively hybridising the sample DNA in a single stranded form to a primer attached to the reaction chamber], wherein a primer is hybridised to the DNA [then to (v))]

[(iv) if the sample DNA has not been attached to a primer attached to the reaction chamber, hybridising a primer to the DNA in a single stranded form]

[(v)] (iv) extending the primer in the presence of a DNA polymerase with a [known] deoxynucleotide [(dNTP)], deoxynucleotide analogue, or dideoxynucleotide [(ddNTP)], wherein the [such] extension is [being] indicated by detection of pyrophsophate [(PPi)] released from the extension reaction; and

(vi) repeating step [(v)] (iv) as required to establish the sequence of the extended primer.

5. [A] The method [according to any one] of claim[s] 1[, 3, or 4], wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

6. [A] The method [according to] of claim 2, wherein the detection step involves labeled terminator.

7. [A] The method claim 1 [-6], wherein the detection of the deoxynucleotide, deoxynucleotide analogue or [/]dideoxynucleotide incorporation is performed in real time.

8. [A] The method [according to any one] of claim[s] 1[-7], wherein the microfluidic device[s] is a disc and [wherein] the fluids [maybe] are moved by centripetal force.

Appendix B

Pending claims as of June 28, 2001

1. A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

(i) incubating the nucleic acid sample with a primer, DNA polymerase, and a deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or a dideoxynucleotide triphosphate;

(ii) measuring the pyrophosphate released in step (i); and

(iii) identifying the nature of the nucleotide base added by measuring which nucleotide caused the release of pyrophosphate in step (ii)

wherein steps (i) to (iii) are performed in a microfluidic device.

2. A method for identifying the sequence of a portion of sample DNA comprising the steps of:

(i) forming immobilised double stranded DNA on one or more reaction areas in a microchannel structure of a microfluidic device;

(ii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase to each of said one or more reaction areas so that extension of primer only occurs if there is a complementarity of the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the strand of sample DNA that is part of the immobilised double stranded DNA; and

(iii) detecting whether or not the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added in step (ii) has been added to the primer DNA in said one or more reaction areas;

(iv) repeating steps (ii) and (iii) as required with a different deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide.

3. A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

(i) attaching 0.1 – 200 pmol of a primer or single stranded DNA sample to each of between one and 100,000 pre-determined areas on the surface of a microfluidic device;

(ii) hybridising small amounts of single stranded sample DNA or primer respectively to each of the predetermined areas;

(iii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase so that extension of the primer only occurs, with consequent release of pyrophosphate, if there is a complementarity with the sample DNA;

(iv) measuring the release of pyrophosphate and from which predetermined area on the device it is released; and

(v) repeating steps (iii) and (iv) as required to construct a DNA sequence for the elongated primers, and hence for portions of the sample DNA.

4. A method for identifying the sequence of a portion of sample DNA, comprising the steps of:

(i) adding sample DNA to a predetermined area on a microfluidic device;

(ii) moving the sample to a reaction chamber on the microfluidic device;

(iii) attaching the sample DNA to a surface of the reaction chamber, wherein a primer is hybridised to the DNA;

(iv) extending the primer in the presence of a DNA polymerase with a deoxynucleotide, deoxynucleotide analogue, or dideoxynucleotide, wherein the

extension is indicated by detection of pyrophsophate released from the extension reaction; and

(vi) repeating step (iv) as required to establish the sequence of the extended primer.

5. The method of claim 1, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

6. The method of claim 2, wherein the detection step involves labeled terminator.

7. The method claim 1, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

8. The method of claim 1, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

9. The method of claim 3, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

10. The method of claim 4, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

11. The method claim 2, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

12. The method of claim 2, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

13. The method claim 3, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

14. The method of claim 3, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

15. The method claim 4, wherein the detection of the deoxynucleotide, deoxynucleotide analogue, dideoxynucleotide, or dideoxynucleotide analogue incorporation is performed in real time.

16. The method of claim 4, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.